REMARKS

Formal Matters

Claims 1-3 and 20 are pending.

Claims 1-3, 16 and 20 were examined and rejected.

Claims 1 and 2 are amended. The amendments to the claims were made solely in the interest of expediting prosecution, and are not to be construed as an acquiescence to any objection or rejection of any claim. Support for the amendments to claims 1 and 2 is found in claim 16 as originally filed, and throughout the specification, in particular at the following exemplary locations: page 24, lines 20-21. Accordingly, no new matter is added.

Claim 16 is canceled without prejudice to renewal, without intent to acquiesce to any rejection, and without intent to surrender any subject matter encompassed by the canceled claims.

Applicants respectfully request reconsideration of the application in view of the remarks made herein.

Interview Summary

The Applicants wish to express their gratitude to Examiners Celsa and Wang for the interview on September 14, 2004, with Applicants' representatives James Keddie, Carol Francis and Jim Diehl.

All current rejections were discussed, as well as arguments to overcome those rejections. Applicants arguments were advanced in the communication filed with the Office on April 2, 2004, and are further elaborated herein.

Rejections under 35 U.S.C. § 103- general discussion

Without wishing to acquiesce to the correctness of this rejection, claims 1 and 2 have been amended to recite a retroviral vector comprising a polynucleotide encoding a GFP having a sequence that of *SEQ ID NO:2*. Since SEQ ID NO:2 sets forth the amino acid sequence of wild-type *Renilla mulleri* GFP, the claims are directed to a retroviral vector comprising a polynucleotide encoding a wild-type *Renilla mulleri* GFP. As suggested by the Examiners during the interview, the claims have also been amended to clarify that the recited vector is for use in a *mammalian* cell.

As discussed in the afore-mentioned interview and as argued in great detail in response to the prior Office Action, the art at the time of filing teaches that a **wild-type** *Aequoria* GFP could **not** be expressed in a mammalian cell using a retroviral vector. This position is factually supported by the papers of Hanazano, Levy, Cheng and Aran, and others[1]. It was not until the amino acid sequence of the *Aequoria* GFP was altered in a significant manner (thereby making it a *mutant* GFP, i.e., *not* wild-type *Aequoria* GFP) that it could be expressed in a mammalian cell using a retroviral vector. The amino acid alterations that result in a retroviral vector-compatible *Aequoria* GFP were neither predictable from the wild-type *Aequoria* sequence, nor trivial to find. Accordingly, several years of research were put into identifying such a GFP and this research resulted in two seminal, back-to-back publications in *Nature Biotechnology* in 1996. As a result of these publications, retroviral vectors encoding **mutant** *Aequoria* GFPs are now very widely used.

The state of the art at the time of filing therefore indicates that wild-type *Aequoria* GFP *could not* be expressed in a mammalian cell using a retroviral vector. One of skill in the art would expect the same to be true of a **wild-type** *Renilla* GFP since the *Aequoria* and *Renilla* proteins have light-emitting activity, i.e., they are both highly fluorescent in cells of their native species, *Aequoria victoria* (a jellyfish) and *Renilla mulleri* (a sea pansy). However, since the wild-type *Aequorea* GFP and wild-type *Renilla* GFP share only about 25% amino acid sequence identity (see Figure 1 of Applicants disclosure), it would have been impossible to determine whether the wild-type *Renilla* GFP contains an amino acid sequence that would make it retroviral-vector compatible. Accordingly, at the time of filing one of skill in the art would expect that a retroviral vector encoding a **wild type** *Renilla mulleri* GFP would be a dismal failure, just like a retroviral vector encoding a **wild type** *Aequoria victoria* GFP.

The Applicants submit that the teachings of Bryan, cited for disclosing the amino acid sequence of wild-type *Renilla mulleri* GFP, would do nothing to increase the expectation of success of a *retroviral vector encoding a* **wild type** *Renilla mulleri* GFP. As would be recognized by one of skill in the art, successful production of a protein using a retroviral vector *at a minimum* requires: a) that the protein is *not* selected against during multiple rounds of cell growth and division, and b) that the protein can be detectably expressed from a vector that is usually present at very low copy number (e.g., a single integrated retroviral vector). Neither of these features could be predicted by the results of Bryan's assay. For example,: a) 8 hours of culture time (the time point at which Bryan's cells were sampled; see col. 103,

[1] See the Response to the prior Office Action.

line 6) is insufficient for cells to undergo multiple rounds of growth and division, and b) Bryan's assay utilizes an autonomously replicating vector having an SV40 origin of replication (see col. 99, lines 50-57), which is known to confer a high copy number to vectors in mammalian cells (see exemplary Exhibits A and B). In view this, the Applicants respectfully submit that Bryan's success in detecting *Renilla mulleri* GFP using a high copy vector system after a mere 8 hours of cell culture would *not* predict successful expression of *Renilla mulleri* GFP using a retroviral vector.

The Applicants respectfully submit that the state of the art therefore taught away from what is being claimed at the time of filing of the instant application. As such, the claimed subject matter could not have been obvious at the time of filing, and, accordingly, this rejection may be withdrawn.

In other words, in stark contrast to the prevailing wisdom at the time of filing, the Applicants have shown that a **wild type** *Renilla mulleri* GFP (i.e., SEQ ID NO:2) *can* be expressed *in a mammalian cell* using *a retroviral vector*. This would have been a surprise in view of the prevailing wisdom at the time of filing, and, as such, would have been quite unexpected by one of skill in the art. Accordingly, this rejection may be withdrawn

The general discussion set forth above supports the Applicants position that Aran, Bryan or Zolutukhin, taken alone or in any combination, cannot render the claimed invention obvious. Withdrawal of rejections that rely on the disclosures of these references is respectfully requested.

Each of the rejections set out in the Office Action is addressed in detail below.

Rejection under 35 U.S.C. § 103 - Bryan and Aran

Claims 1-3 and 16 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Bryan and Aran. The Office Asserts that Bryan's GFP, in combination with Aran's retroviral vectors, renders the subject matter of the instant claims obvious. This rejection is respectfully traversed.

As discussed above, Bryan and Aran, either taken alone or in combination, cannot render the claimed invention obvious because one of skill in the art at the time of filing would have no reasonable expectation of success in expressing a wild-type *Renilla mulleri* GFP in a mammalian cell using a retroviral vector. For example, Aran, as cited in this rejection, states that expression of *wild-type Aequorea* GFP in a mammalian cell from a retroviral vector did not work (see first full paragraph of page 204).

Accordingly, the Applicants respectfully submit that a *prima facie* case of obviousness has not been established, and this rejection should be withdrawn.

Rejection under 35 U.S.C. § 103 - Aran and Bryan

Claims 1-3, 16 and 20 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Aran and Bryan. The Office Asserts that Aran's retroviral vectors, in combination with Bryan's GFP, renders the subject matter of the instant claims obvious.

As discussed above, Bryan and Aran, either taken alone or in combination, cannot render the claimed invention obvious because one of skill in the art at the time of filing would have no reasonable expectation of success in expressing a wild-type *Renilla mulleri* GFP in a mammalian cell using a retroviral vector. For example, Aran, as cited in this rejection, states that expression of *wild-type Aequorea* GFP in a mammalian cell from a retroviral vector did not work (see first full paragraph of page 204).

Accordingly, the Applicants respectfully submit that a *prima facie* case of obviousness has not been established, and this rejection should be withdrawn.

Rejection under 35 U.S.C. § 103 - Aran, Bryan and Zolutukhin

Claim 20 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Aran, Bryan and Zolutukhin. The Office Asserts that Aran's retroviral vectors, Bryan's Renilla GFP and Zolutukhin's human codon optimized GFP renders the subject matter of the instant claims obvious.

As discussed above, Bryan and Aran, either taken alone or in combination, cannot render the claimed invention obvious because one of skill in the art at the time of filing would have no reasonable expectation of success in expressing a wild-type *Renilla mulleri* GFP in a mammalian cell using a retroviral vector.

Zolutukhin is cited to provide a human codon optimized *Aequoria* GFP. However, according to Hanazono (Hum. Gene Ther. 1997, 8:1313-9), even a human codon optimized *Aequoria* GFP could not be expressed in a mammalian cell using a retroviral vector (see, "many attempts by our laboratory to isolate stable retroviral producer cell clones secreting biologically active vectors containing either the highly fluorescent S65T-GFP mutant or humanized GFP have *failed*" (emphasis added)). Accordingly,

Zolutukhin does not meet the deficiencies of Bryan and Aran, and does nothing to increase the expectation of success of the claimed vector.

Accordingly, Applicants respectfully submit that a *prima facie* case of obviousness has not been established, and this rejection should be withdrawn.

Rejection under 35 U.S.C. § 103 - Zolutukhin and Bryan

Claims 1-3, 16 and 20 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zolutukhin and Bryan. The Office Asserts that Zolutukhin's human codon optimized GFP retroviral vector, in combination with Bryan's *Renilla* GFP, renders the subject matter of the instant claims obvious.

As discussed in detail above, at the time of filing of the instant application, there was no reasonable expectation of success in expressing a wild type *Renilla mulleri* GFP in a mammalian cell using a retroviral vector. This assertion is supported by the literature at the time of filing of the instant application. In particular, Hanazono, Aran, Levy and Cheng, and others, as discussed above, indicate that several research groups independently experienced great difficulty in using a retroviral vector encoding wild type *Aequorea* GFP. Until the work was actually done, there was no reasonable expectation of success of producing wild type *Renilla* GFP in a mammalian cell using a retroviral vector

The arguments presented in detail above apply with equal force to the rejection of the claims based upon the combination of Zolutukhin and Bryan. Thus this rejection should also be withdrawn because the references provide no reasonable expectation of success in the practice of the invention given the state of the art at the time of filing. Zolutukhin or Bryan's statements about the desirability of *Renilla* GFP as a reporter for mammalian cells do not provide any reasonable expectation of success.

Accordingly, the Applicants respectfully submit that Zolutukhin and Bryan cannot be combined to render the claimed invention obvious, and this rejection should be withdrawn.

CONCLUSION

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number RIGL-011.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: September 30, 2004

By: _____
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Enclosures: Exhibits A-B

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09/710,058 Exhibit A

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1: J Virol. 1991 Nov;65(11):5944-51. Related Article

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Regulated replication of an episomal simian virus 40 origin plasmid in COS7 cells.

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The replication of a simian virus 40 (SV40) origin-containing plasmid, pSLneo, stably transfected CO cells has been studied. pSLneo contains the SV40 origin of replication and encodes the positive selecta marker for G418 resistance. In transient replication assays, pSLneo replicates to a high copy number ir COS7 cells. Uncontrolled SV40 plasmid replication has been reported to be lethal to such transfected Thus, it was anticipated that extensive plasmid replication would preclude isolation of permanent cell containing pSLneo. However, significant number of G418-resistant colonies arose after transfection of COS7 cells with pSLneo. Cell lines established from these drug-resistant colonies contained between and 1,000 extrachromosomal pSLneo copies per cell. Episomal plasmid DNA in pSLneo/COS7 lines v stably maintained after 2 months of continuous culture in selective medium. Bromodeoxyuridine label and density shift experiments demonstrated that replication of pSLneo closely paralleled that of cellula DNA. On average, plasmid DNA did not replicate more than once during a single cell generation peric Regulation of pSLneo replication appeared to be negatively controlled by a cis-acting mechanism. Endogenous copies of episomal pSLneo remained at a stable low copy number during the simultaneou high-level replication of a newly transfected plasmid encoding SV40 large T antigen in the same cells. These results indicate that regulated replication of an SV40 origin plasmid can be acquired in a cell an not require the presence of additional genetic elements. The molecular mechanism by which cells enfo this regulation on extrachromosomal SV40 plasmids remains to be defined.

mammalian cells.

PMID: 1656081 [PubMed - indexed for MEDLINE]

09/710,058 Exhibit B

1: Proc Natl Acad Sci U S A. 1997 Jun 10;94(12):6450-5. Related Article

Safety-modified episomal vectors for human gene therapy.

Cooper MJ, Lippa M, Payne JM, Hatzivassiliou G, Reifenberg E, Fayazi B, Perales JC, Morriso Templeton D, Piekarz RL, Tan J.

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The effectiveness of ongoing gene therapy trials may be limited by the expression characteristics of vi and plasmid-based vectors. To enhance levels of heterologous gene expression, we have developed a s modified episomal expression vector that replicates extrachromosomally in human cells. This vector s employs a simian virus 40 (SV40) large T antigen mutant (107/402-T) that is deficient in binding to h tumor suppressor gene products, including p53, retinoblastoma, and p107, yet retains replication competence. These SV40-based episomes replicate to thousands of copies by 2-4 days after gene trans multiple types of human cell lines, with lower activity in hamster cells, and no detectable activity in dc and murine cell lines. Importantly, 107/402-T has enhanced replication activity compared with wild-ty antigen; this finding may be due, in part, to the inability of p53 and retinoblastoma to inactivate 107/4(function. We demonstrate that the level and duration of 107/402-T expression regulates the observed episomal copy number per cell. Compared with standard plasmid constructs, episomes encoding 107/4 yield approximately 10- to 100-fold enhanced levels of gene expression in unselected populations of transient transfectants. To determine if 107/402-T-based episomes replicate extrachromosomally in vi tumor explants in nude mice were directly injected with liposome/DNA complexes. Using a PCR-base assay, we demonstrate that SV40-based episomes replicate in human cells after direct in vivo gene trar These data suggest that safety-modified SV40-based episomes will be effective for cancer gene therap because high level expression of therapeutic genes in transient transfectants should yield enhanced tur elimination.

MeSH Terms:

- Animals
- Antigens, Polyomavirus Transforming/biosynthesis
- Antigens, Polyomavirus Transforming/genetics
- Cell Line
- Comparative Study
- DNA Primers
- DNA Replication
- Dogs
- Gene Therapy/methods*
- Gene Therapy/standards
- Genes, Tumor Suppressor
- Genetic Vectors*
- Hamsters
- Human
- Kinetics
- Luciferase/biosynthesis
- Mice
- Polymerase Chain Reaction
- Protein p53/metabolism
- Rats
- Recombinant Proteins/biosynthesis
- Retinoblastoma Protein/metabolism
- Simian virus 40/genetics